

B800→B850 Energy Transfer Mechanism in Bacterial LH2 Complexes Investigated by B800 Pigment Exchange

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ABSTRACT Femtosecond transient absorption measurements were performed on native and a series of reconstituted LH2 complexes from *Rhodospseudomonas acidophila* 10050 at room temperature. The reconstituted complexes contain chemically modified tetrapyrrole pigments in place of the native bacteriochlorophyll *a*-B800 molecules. The spectral characteristics of the modified pigments vary significantly, such that within the B800 binding sites the B800 Q_y absorption maximum can be shifted incrementally from 800 to 670 nm. As the spectral overlap between the B800 and B850 Q_y bands decreases, the rate of energy transfer (as determined by the time-dependent bleaching of the B850 absorption band) also decreases; the measured time constants range from 0.9 ps (bacteriochlorophyll *a* in the B800 sites, Q_y absorption maximum at 800 nm) to 8.3 ps (chlorophyll *a* in the B800 sites, Q_y absorption maximum at 670 nm). This correlation between energy transfer rate and spectral blue-shift of the B800 absorption band is in qualitative agreement with the trend predicted from Förster spectral overlap calculations, although the experimentally determined rates are ~5 times faster than those predicted by simulations. This discrepancy is attributed to an underestimation of the electronic coupling between the B800 and B850 molecules.

INTRODUCTION

The recent crystal structure of the LH2 complex from *Rps. acidophila* 10050 at a resolution of 2.5 Å (McDermott et al., 1995) has provided an excellent opportunity to study excitation energy transfer in great detail (for review, see Sundström et al., 1999). The LH2 complex has a nonameric ring structure, with each monomeric unit containing an α - and β -apoprotein, three BChl molecules, and one carotenoid (Arellano et al., 1998) molecule. The nine α -apoproteins form a hollow cylinder with the nine β -apoproteins arranged concentrically outside. Eighteen BChl molecules are sandwiched between the α - and β -apoprotein helices and form a continuous overlapping ring. These molecules absorb mainly at ~850 nm and are called the BChl-B850 molecules, with B850 denoting the binding site according to the Q_y absorption maximum of the native BChl within. A further nine BChl molecules are located more peripherally in the complex and are situated between the β -apoproteins.

These molecules absorb light at ~800 nm and are correspondingly denoted the BChl-B800 molecules. In addition, the carotenoids span the membrane, making van der Waals contact with both the BChl-B800 and -B850 molecules.

We are particularly interested in trying to understand the mechanism of energy transfer between the BChl-B800 and BChl-B850 molecules. The kinetics of this process have been extensively studied. B800→B850 energy transfer was first clearly resolved in *Rb. sphaeroides* by Shreve et al., where femtosecond transient absorption measurements revealed a time constant of 0.7 ps at room temperature (Shreve et al., 1991). This result has been confirmed in further transient absorption (Hess et al., 1995; Monshouwer et al., 1995; Kennis et al., 1997; Pullerits et al., 1997) and fluorescence upconversion studies (Jimenez et al., 1996). For comparison, the time constant for B800→B850 energy transfer in *Rps. acidophila* 10050 is 0.9 ps (Kennis et al., 1997; Ma et al., 1997).

B800→B850 energy transfer has been generally thought to occur by the Förster dipole-dipole weak interaction mechanism (van Grondelle et al., 1994). The variable parameters include the geometry of the transition dipole moments involved, the center-to-center distance between donor and acceptor molecules (treated as point dipoles), the refractive index of the intervening pigment environment, and the overlap integral between the emission spectrum of the donor and the absorbance spectrum of the acceptor molecules (Förster, 1948). To test whether or not B800→B850 energy transfer occurs by this mechanism, it is necessary to systematically alter one of the above parameters (keeping the other parameters constant) and then compare the effect of this alteration on the actual rate of energy transfer with that predicted by the theory.

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J. L. Herek, N. J. Fraser, and T. Pullerits contributed equally to this paper. Abbreviations used: LH, light-harvesting; A_x , absorbance at x nm; BChl, bacteriochlorophyll *a* (esterified with phytol); BChl_{gg}, BChl esterified with geranylgeraniol at position C17³ (the abbreviations for the other pigments are summarized in Fig. 1); BChl-B800, -B850, BChl molecule in the B800, B850 binding pockets, respectively; XXX-B800, general reference to any native or modified (bacterio)chlorophyll pigment (XXX) in the B800 binding sites; B800→B850, energy transfer from the XXX-B800 to the BChl-B850 molecules; LM, n-dodecyl- β -D-maltoside; *Rb.*, *Rhodobacter*; RC, reaction center; *Rps.*, *Rhodospseudomonas*.

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The variable that can be altered most easily is the spectral overlap function. For example, LH2 complexes in which the Q_y absorption maximum of either the BChl-B800 or -B850 molecules is blue-shifted have been created by site-directed mutagenesis (Fowler et al., 1992, 1997). In these mutants the changes in the rate of B800→B850 energy transfer were in qualitative agreement with those predicted by Förster theory (Hess et al., 1994; Fowler et al., 1997). The spectral separation between the BChl-B800 and -B850 Q_y absorption transitions can also be varied by changing either the pressure (Tars et al., 1994; Wu et al., 1997) or temperature (Pullerits et al., 1997). On cooling, the spectral overlap between the BChl-B800 and -B850 Q_y transitions decreases due to a narrowing of both bands and a pronounced red-shift in the BChl-B850 band. The time constants for B800→B850 energy transfer in *Rb. sphaeroides* at 300, 77, and 4.2 K are 0.7, 1.2, and 1.5 ps, respectively (Pullerits et al., 1997).

Model calculations based solely upon Förster theory, which also took into account the overlap due to the high-energy vibrational states, suggested significantly slower rates than were experimentally measured. Two possible explanations were given for this anomaly. It was suggested that the BChl-B800 molecules transfer their excitation energy into the higher-energy side of the exciton manifold associated with the BChl-B850 molecules. This absorption component arises from the mixing of the excited states of the BChl-B850 molecules and is thought to be located in the same spectral region as the Q_y absorption band of the BChl-B800 molecules (Wu et al., 1996; Sauer et al., 1996; Kühn and Sundström, 1997; Koolhaas et al., 1998; Bandilla et al., 1998; Sundström et al., 1999; Leupold et al., 1999). Alternatively, enhancement of the electronic coupling due to the higher-order coupling terms and involving a superexchange mechanism via carotenoids and/or other nearby chromophores may facilitate rapid B800→B850 energy transfer (Pullerits et al., 1997; Scholes et al., 1997; Damjanovic et al., 1998; Krueger et al., 1998, 1999).

The spectroscopic properties of certain LH2 complexes can be radically altered by selectively exchanging the native BChl molecules in the B800 binding sites with chemically modified tetrapyrrole pigments (Bandilla et al., 1998; Fraser, 1998; Fraser et al., 1999). In this approach, the BChl-B800 molecules are released from their binding pockets by an acid treatment. '93B850-only'94 complexes can then be purified by ion-exchange chromatography, and finally the empty binding sites can be reconstituted with either native or modified (bacterio)chlorin molecules (Bandilla et al., 1998; Fraser, 1998; Fraser et al., 1999). Thorough characterization studies have shown that the overall structures of the reconstituted complexes are not affected by the pigment-exchange procedure (Fraser et al., 1999). In addition, the reconstituted pigments are correctly bound within the B800 binding pockets (Gall et al., 1999) and

participate in efficient energy transfer to the BChl-B850 molecules (Bandilla et al., 1998; Fraser et al., 1999).

Here, we present measurements of the rate of B800→B850 energy transfer in a series of complexes reconstituted with different (B)Chl derivatives such that the Q_y absorption band of the (B)Chl-B800 molecules is progressively blue-shifted. The validity of the Förster mechanism in describing the observed kinetics is discussed and alternative mechanisms are considered.

MATERIALS AND METHODS

Isolation of LH2 complexes

Liquid cultures of *Rps. acidophila* 10050 were grown anaerobically at 30°C in Pfenning's medium (Pfenning, 1969). Cells were harvested by centrifugation. LH2 complexes were prepared as described by Fraser et al. (1999).

Pigment-exchange procedure

The pigment-exchange procedure has been detailed elsewhere (Fraser, 1998). In short, all of the BChl-B800 molecules can be released from their binding sites by incubating an LH2 sample (nonamer concentration of 0.7 μ M) in buffer containing Triton TBG10 at a pH of 4.75 at 30°C for 1 h. B850-only complexes were then purified by ion-exchange chromatography using phosphocellulose as adsorbent. Later, the reconstituted complexes were made by incubating a B850-only sample containing 0.1% (w/v) LM with a 3-fold excess of the desired pigment (see Fig. 1) for 2 h at pH 8 and at room temperature.

Pigments: in vitro characterization

The fluorescence lifetimes of the modified pigments were measured via the time-correlated single photon counting technique. Excitation was in either the Soret or Q_y bands with 200-fs pulses generated by a Ti:Sapphire laser, pulse picked at a repetition rate of 800 kHz. Fluorescence was detected at magic angle (54.7°) using a microchannel plate photomultiplier. The response function was 120 ps. The fluorescence lifetimes of the pigments are all on the nanosecond time scale (Table 1) and, as such, their intramolecular photophysical dynamics will have negligible effect on the picosecond B800→B850 energy transfer times.

The fluorescence emission spectrum of each pigment was measured using a standard fluorometer with photomultiplier detection. All measurements were made in a 1-cm quartz cuvette, with diethylether as solvent. The optical density was kept well below 0.1 at the Q_y absorption maximum to prevent self-absorption and pigment aggregation.

Steady-state absorption measurements

Absorption spectra were measured using a standard spectrophotometer. All measurements were made in a 1-cm quartz cuvette. The samples had an A_{850} of 0.5.

Transient absorption measurements

The femtosecond spectrometer used here is based on an amplified Ti:Sapphire laser system, producing ~100 fs pulses at 790 nm with an average output power of 1 W and a repetition rate of 5 kHz. Part of this light was used to pump an optical parametric amplifier for creating exci-

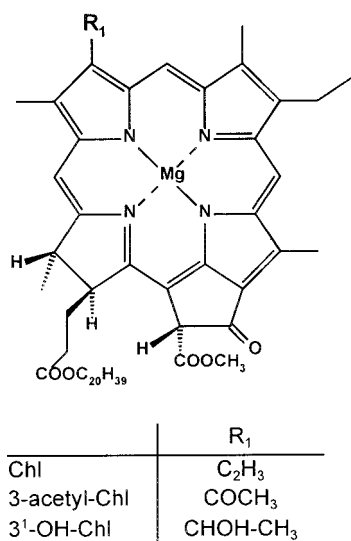
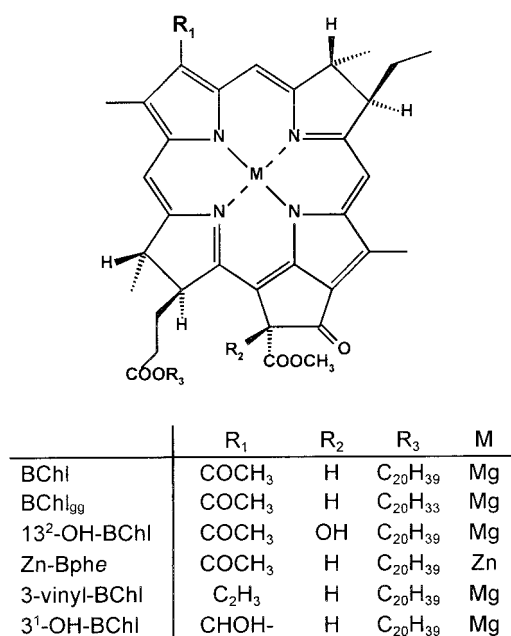


FIGURE 1 Structures of (B)Chl pigments introduced into the reconstituted complexes.

tation pulses in the 660–790-nm region. The remaining light was focused into a 1-cm sapphire plate to generate a white-light continuum that was used for probing. The relative polarization of the pump and probe pulses was set to the magic angle (54.7°). The instrument response function, measured as the cross-correlation of the pump and probe pulses at the sample position, had a Gaussian time profile with a full-width at half-maximum (fwhm) of ~130 fs. To avoid nonlinear singlet-singlet and singlet-triplet excitation annihilation and prevent sample degradation, the excitation intensity was attenuated to $\sim 5 \times 10^{13}$ photons/cm². All measurements were made in a 2-mm rotating quartz cell. The samples were diluted with the appropriate buffer solution such that $A_{\text{exc}} \sim 0.5$. Absorption spectra of each sample were recorded before and after the transient absorption measurements to check that no degradation had occurred.

TABLE 1 Fluorescence lifetimes of the pigment molecules in diethylether

Pigment	τ (ns)	Reference
Bchl	2.9 ± 0.1*	This work
	3.0 ± 0.2	†‡
BChl _{gg}	2.9 ± 0.1*	This work
13 ² -OH-BChl	2.4 ± 0.1*	This work
Zn-Bphe	2.6 ± 0.1	This work
	2.6 ± 0.2	†
3-vinyl-BChl	3.0 ± 0.1	This work
	3.1 ± 0.2	‡
3 ¹ -OH-BChl	3.1 ± 0.1	This work
	2.9 ± 0.2	‡
3-acetyl-Chl	5.7 ± 0.1	This work
	5.9 ± 0.2	‡
Chl	5.8 ± 0.1	This work
	6.0 ± 0.2	‡

All pigments were excited in their Soret band. An asterisk (*) indicates additional measurements with Q_y excitation; in these cases the lifetime represents an average of the measured values.

†Teuchner et al., 1997.

‡Teuchner et al., 1994.

RESULTS

The absorption spectra of native LH2, B850-only, and selected reconstituted complexes are shown in Fig. 2. The BChl-B800 Q_y absorption band is absent in the B850-only complex (Fig. 2 *b*) but is restored in the BChl-B800 recon-

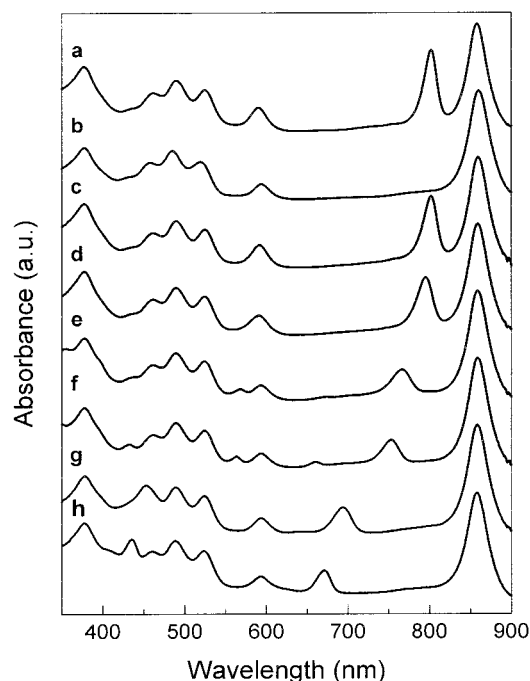


FIGURE 2 Absorption spectra of the (a) native, (b) B850-only, and (c) BChl-, (d) Zn-Bphe, (e) 3-vinyl-BChl-, (f) 3¹-OH-Bchl-, (g) 3-acetyl-Chl-, and (h) Chl-B800 reconstituted complexes.

stituted complex. The absorption spectra of the complexes reconstituted with BChl_{gg}- and 13²-OH-BChl-B800 are the same as those for the BChl-B800 reconstituted complex (data not shown). In the complexes reconstituted with Zn-Bphe-, 3-vinyl-BChl-, 3¹-OH-BChl-, 3-acetyl-Chl-, and Chl-B800, the (B)Chl-B800 Q_y absorption maximum is progressively blue-shifted (Fig. 2, *d-h*; Table 2). The small shoulder at 680 nm in the absorption spectrum of the 3¹-OH-BChl-B800 reconstituted complex is due to a 3¹-OH-Chl oxidation product. As the (B)Chl-B800 Q_y transition is blue-shifted, the spectral overlap between the (B)Chl-B800 and BChl-B850 molecules progressively decreases. This allowed the effect of spectral overlap on the rate of B800→B850 energy transfer to be assessed over a large wavelength range (650–800 nm).

In each of the reconstituted complexes the rate of B800→B850 energy transfer in the various complexes was determined by monitoring the time-dependent rise in bleaching of the BChl-B850 Q_y absorption band at 870 nm after exciting its (B)Chl-B800 Q_y transition. The excitation wavelengths were tuned to ~10 nm to the blue of the absorption maxima (Table 2) to allow complementary measurements of the recovery of the B800 ground-state absorption. These results verified the time constants obtained from the rise kinetics of the B850 bleach (results not shown). The measured kinetics for the native and selected reconstituted complexes are shown in Fig. 3 and summarized in Table 2. In all cases, the rise of the B850 bleach is characterized by two components: an instantaneous (pulse-limited) signal (between 1 and 25% of the total amplitude; vide infra) and a slower, variable signal. The latter component is due to the arrival of excitation energy from the (B)Chl-B800 molecules and was fitted to a single exponential function convoluted with the instrument response function. For all of the samples, the BChl-B850 bleach decays in ~800 ps. In the native complex, B800→B850 energy transfer takes ~0.9 ps. This value is consistent with previous measurements

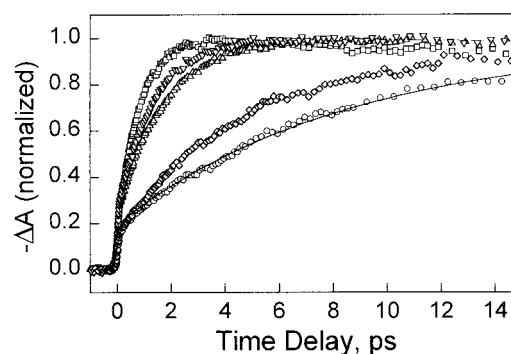


FIGURE 3 The rise in bleaching of the Q_y absorption band of the BChl-B850 molecules with time in native and selected reconstituted complexes. The excitation wavelengths are recorded in Table 2. B850 bleaching was probed at 870 nm. The time constants for B800→B850 energy transfer are summarized in Table 2. Key: \square , native; ∇ , 3-vinyl-BChl-; \triangle , 3¹-OH-BChl-; \diamond , 3-acetyl-Chl-; and \circ , Chl-B800-reconstituted complexes.

(Ma et al., 1997; Kennis et al., 1997). Similarly, in the BChl-B800 reconstituted complex, the B800→B850 energy transfer takes 0.9 ps; this time constant was also found for those complexes containing pigments with similar absorption, viz. BChl_{gg} or 13²-OH-BChl. This observation serves as further confirmation that the reconstituted pigments adopt the same conformation as those in the native complex. In the 3-vinyl-BChl-, 3¹-OH-BChl-, 3-acetyl-Chl-, and Chl-B800 reconstituted complexes, the rate of B800→B850 energy transfer decreases as the spectral separation between the (B)Chl-B800 and BChl-B850 Q_y absorption bands increases (Fig. 3 and Table 2). In the furthest blue-shifted, Chl-B800 reconstituted complex, the time constant for B800→B850 energy transfer is 8.3 ps. This corresponds to a 10-fold decrease in the rate of energy transfer compared with that in native LH2.

The origin of the instantaneous component in the rise kinetics was investigated in a control experiment using the B850-only complex. The B850-only sample was excited at the same excitation wavelength used in the measurements with the 3-acetyl-Chl-B800 reconstituted complex ($\lambda_{\text{exc}} = 685$ nm). Instantaneous bleaching of the B850 Q_y absorption band was observed (results not shown), indicating direct excitation of the BChl-B850 molecules, even at this short wavelength.

DISCUSSION

If the structure of a donor-acceptor system is known and the electronic coupling can be evaluated (as in LH2), then it proves convenient to express the Förster transfer rate via an overlap integral of normalized absorption and fluorescence spectra and the donor-acceptor interaction energy. Starting from Fermi's golden rule one can obtain the following

TABLE 2 Q_y absorption maxima of the (B)Chl-B800 molecules in native and reconstituted LH2 complexes and the time constants for B800→B850 energy transfer

B800 site			
Pigment	$\lambda_{\text{max}}^{\text{abs}}$ (nm)	λ_{exc} (nm)	τ (ps)
Native LH2			
BChl	800	785	0.9 ± 0.1
(B)Chl-B800 reconstituted complexes			
BChl	800	785	0.9 ± 0.1
BChl _{gg}	800	785	0.8 ± 0.1
13 ² -OH-BChl	800	785	0.8 ± 0.1
Zn-Bphe	794	785	0.8 ± 0.1
3-vinyl-BChl	765	754	1.4 ± 0.2
3 ¹ -OH-BChl	753	742	1.8 ± 0.2
3-acetyl-Chl	694	685	4.4 ± 0.5
Chl	670	660	8.3 ± 0.5

equation (Agranovich and Galanin, 1982):

$$k_{i \rightarrow j} = \frac{4\pi^2}{h^2 c} V^2 \int \left[\frac{F_i(\nu)}{\nu^3} \right] \left[\frac{A_j(\nu)}{\nu} \right] d\nu$$

$$= 1.18 V^2 \Theta \quad (1)$$

where $k_{i \rightarrow j}$ is the rate of energy transfer between the donor (i) and acceptor (j) molecules (units ps^{-1}), V is the dipole-dipole interaction between them (cm^{-1}) and Θ is the overlap integral between the donor fluorescence spectrum $F_i(\nu)$ divided by ν^3 and acceptor absorption spectrum $A_j(\nu)$ divided by ν for which the intensities (area) have been normalized to unity on the cm^{-1} scale (Pullerits et al., 1997).

Here we have calculated the rate of B800→B850 energy transfer in each of the reconstituted complexes in order to explore the effect of increased spectral separation between the donor fluorescence and acceptor absorption maxima (energy gap) on the kinetics of excitation transfer. Overlap integrals were calculated using the absorption spectrum of the B850-only complex (Fig. 2 *b*) and the fluorescence emission spectra of the modified pigments (for example, see inset of Fig. 4). To account for the Stokes shift of the emission band in the complexes, the emission spectra of the modified pigments were shifted 80 cm^{-1} from the absorption maxima, based on B800 fluorescence in native LH2 complexes (De Caro et al., 1994). The Q_x band of the B850 absorption spectrum was fitted to a Gaussian function and subtracted before modeling. Elsewhere, the B800-B850 interaction (V) in the native complexes has been estimated to be $\sim 30 \text{ cm}^{-1}$ (Krueger et al., 1998; Pullerits et al., 1997). In our reconstituted complexes, however, the donor molecules are chemically different, which affects the strength of the coupling between the (B)Chl-B800 and BChl-B850 molecules. We have estimated the transition dipole mo-

ments of the 3-vinyl-BChl, 3¹-OH-BChl, 3-acetyl-Chl, and Chl pigments relative to BChl using their emission spectra, their radiative rates (Teuchner et al., 1994), and the ν^3 relationship between Einstein A and B coefficients. Our calculations indicate that the square of the B800-B850 interaction in the complexes reconstituted with 3-vinyl-BChl, 3¹-OH-BChl, 3-acetyl-Chl, and Chl is ~ 0.88 , 0.92 , 0.74 , and 0.52 times that in LH2, respectively. For each complex, the value of V^2 in Eq. 1 was scaled accordingly. It is worth mentioning that there is a further complication with the dielectric constant if one is using a conventional Förster equation with molar extinction coefficient and natural lifetime (Moog et al., 1984). We do not discuss this point further because Eq. 1 includes the refractive index in the interaction V .

The calculated energy transfer times, together with the experimentally measured data points, are presented in Fig. 4. Error bars of 15% are added to the calculated data, allowing for signal-to-noise limitations in the emission spectra and uncertainties in estimating V values and the Stokes shift. The qualitative trends of the measured and calculated transfer times agree well. Nonetheless, the calculated transfer rates in all of the reconstituted complexes are about five times slower than those that were experimentally measured: a discrepancy noted previously in *Rb. sphaeroides* (Pullerits et al., 1997).

According to Eq. 1 there can be two reasons for this discrepancy: either the spectral overlap integral Θ or the interaction energy V (or both) have been underestimated. Previously, it has been speculated that the high-energy part of the B850 exciton manifold contributes to Θ (Braun and Scherz, 1991; Wu et al., 1996; Pullerits et al., 1997; Koolhaas et al., 1998; Leupold et al., 1999). It seems unlikely that additional spectral components can make a significant

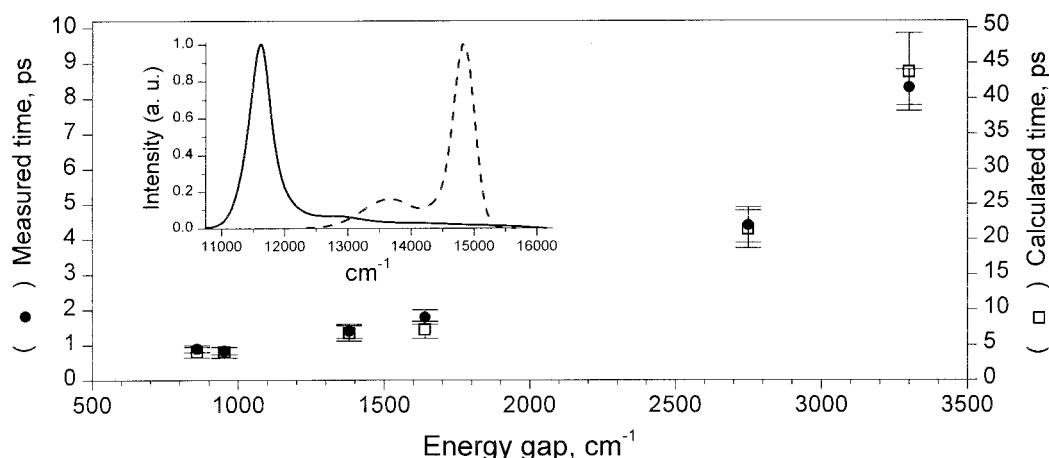


FIGURE 4 Experimental (solid circles) and calculated (open squares) energy gap dependence of B800→B850 energy transfer. The open squares correspond to the absolute energy transfer times, as calculated according to Eq. 1. Note the different scaling of the two data sets; the calculated times are ~ 5 times greater than those measured experimentally. The absorption spectrum of the B850-only complexes (solid line) and emission spectrum of Chl (dashed line) used in the spectral overlap calculation are shown in the inset. The Q_x band of the B850 absorption was subtracted.

contribution to our calculated spectral overlap integral, as our simulation uses the actual B850-only absorption spectrum (which includes all the excitonic and vibronic manifolds) and the emission spectrum of each pigment used for reconstitution. This leaves us to consider additional contributions to the B800-B850 interaction energy. An interaction of $\sim 65 \text{ cm}^{-1}$, rather than the $\sim 30 \text{ cm}^{-1}$ as estimated from simple dipole-dipole coupling, would account for the observed difference between measured and calculated transfer times. It has been suggested previously that the carotenoids contribute to the coupling between the (B)Chl-B800 and BChl-B850 molecules (Pullerits et al., 1997; Scholes et al., 1997; Damjanovic et al., 1998; Krueger et al., 1998). More recently, calculations by Krueger et al. (1999) indicate that the carotenoids strengthen the interaction between the B800 and B850 molecules by $10\text{--}20 \text{ cm}^{-1}$. The results presented here are consistent with this theoretical prediction.

Regarding alternative mechanisms, it has been pointed out that non-diagonal (Kühn and Sundström, 1997) and/or diagonal (Sumi, 1999) electron-phonon coupling may facilitate efficient excitation transfer to optically forbidden exciton levels. If these couplings were the source of the disagreement between the calculated and measured transfer times, we would expect the mismatch to be larger for those (B)Chl-B800 Q_y bands that lie within the exciton manifold of the B850 molecules (i.e., at wavelengths down to $\sim 750 \text{ nm}$). As the difference between the calculated and measured times in all of the complexes is a factor of five, such coupling terms cannot be responsible for the discrepancy.

Finally, a brief discussion of the B850-only absorption spectrum. We draw the reader's attention to the very long tail of the B850 Q_y absorption band even beyond $14,500 \text{ cm}^{-1}$ (see inset of Fig. 4). Neither fluorescence line narrowing (Renge et al., 1987; Peterman et al., 1997) nor spectral hole-burning (Gillie et al., 1989) (both at low temperatures) have revealed any modes above 1800 cm^{-1} with significant coupling to the Q_y electronic transition. Furthermore, because the modes above $\sim 1000 \text{ cm}^{-1}$ have very small Huang-Rhys factors, absorption in which two vibrational quanta are created is negligible. Together, this means that the vibronic manifold associated with the main B850 band has very low intensity in this region. There is accumulating evidence that the nearest-neighbor interaction among BChl-B850 molecules is $\sim 300 \text{ cm}^{-1}$ (Koolhaas et al., 1998; Scholes et al., 1999), which puts the higher edge of the B850 exciton manifold at $\sim 12,700 \text{ cm}^{-1}$. These higher-energy excitonic states of B850 have their own vibronic manifolds, which seems to be the most likely origin of the B850-related absorption above $14,500 \text{ cm}^{-1}$. Recall also that after exciting any of the (B)Chl-B800 bands, an instantaneous component in the B850 bleach was always observed (see Fig. 3). Here, we suggest that the vibronic manifold related to the upper band edge of B850 is the most likely explanation for the instantaneous component in the B850 bleach. Vibronic coupling in excitonic systems is a

non-trivial issue and will be addressed in future work, when we plan to systematically study the origin of the B850-related background absorption at high energies.

CONCLUSIONS

The correlation between B800→B850 energy transfer rate and spectral blue-shift of the B800 absorption band is in qualitative agreement with the trend predicted from Förster spectral overlap calculations, although the experimentally determined rates are considerably higher than those predicted by simulations. We believe the most likely explanation for the fivefold discrepancy between the calculated and measured transfer rates to be an underestimation of the donor-acceptor interaction energy, and suggest that additional contributions to this term may come from the carotenoids and/or other chromophores. Further experiments are planned to test specifically for carotenoid involvement in B800→B850 energy transfer, using carotenoid-deficient LH2 complexes such as those described by Todd et al. (1998).

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REFERENCES

- Agranovich, V. M., and M. D. Galanin. 1982. Electronic excitation energy transfer in condensed matter. North-Holland, Amsterdam.
- Arellano, J. B., B. B. Raju, K. R. Naqvi, and T. Gillbro. 1998. Estimation of pigment stoichiometries in photosynthetic systems of purple bacteria: special reference to the (absence of) second carotenoid in LH2. *Photochem. Photobiol.* 68:84–87.
- Bandilla, M., B. Ücker, M. Ram, I. Simonin, E. Gelhaye, G. McDermott, R. J. Cogdell, and H. Scheer. 1998. Reconstitution of the B800 bacteriochlorophylls in the peripheral light harvesting complex B800–850 of *Rhodospirillum rubrum* 2.4.1 with BChl a and modified (bacterio-) chlorophylls. *Biochim. Biophys. Acta.* 1364:390–402.
- Braun, P., and A. Scherz. 1991. Polypeptides and bacteriochlorophyll organization in the light-harvesting complex-B850 of *Rhodospirillum rubrum* R-26.1. *Biochemistry.* 30:5177–5184.
- Damjanovic, A., T. Ritz, and K. Schulten. 1998. Light-harvesting and photoprotection by carotenoids in photosynthesis. *Biophys. J.* 74:76a. (Abstr.).
- De Caro, C., R. W. Visschers, R. van Grondelle, and S. Völker. 1994. Inter- and intraband energy transfer in LH2-antenna complexes of purple bacteria. A fluorescence line-narrowing and hole-burning study. *J. Phys. Chem.* 98:10584–10590.
- Förster, Th. 1948. Intermolecular energy transfer and fluorescence. *Annu. Rev. Phys.* 2:55–75.
- Fowler, G. J. S., S. Hess, T. Pullerits, V. Sundström, and C. N. Hunter. 1997. The role of beta Arg(-10) in the B800 bacteriochlorophyll and carotenoid pigment environment within the light-harvesting LH2 complex of *Rhodospirillum rubrum*. *Biochemistry.* 36:11282–11291.

- Fowler, G. J. S., R. W. Visschers, G. G. Grief, R. van Grondelle, and C. N. Hunter. 1992. Genetically modified photosynthetic antenna complexes with blueshifted absorbance bands. *Nature*. 355:848–850.
- Fraser, N. J. 1998. Modified pigments and mechanisms of energy transfer in LH2 complexes from purple bacteria. Ph.D. thesis, University of Glasgow, UK.
- Fraser, N. J., P. J., Dominy, B. Ücker, I. Simonin, H. Scheer, and R. J. Cogdell. 1999. Selective release, removal and reconstitution of Bchl_a molecules into the B800 sites of LH2 complexes from *Rhodospseudomonas acidophila* 10050. *Biochemistry*. 38:9684–9692.
- Gall, A., N. J. Fraser, M.-C. Bellissent-Funel, H. Scheer, B. Robert, and R. J. Cogdell. 1999. Bacteriochlorin-protein interactions in native B800–B850, B800 deficient, and B800-Bchl_a reconstituted complexes from *Rhodospseudomonas acidophila*, strain 10050. *FEBS Lett.* 449: 269–272.
- Gillie, J. K., G. J. Small, and J. H. Golbeck. 1989. Non-photochemical hole burning on the native antenna complex of PSI (PSI-200). *J. Phys. Chem.* 93:1620–1627.
- Hess, S., E. Åkesson, R. J. Cogdell, T. Pullerits, and V. Sundström. 1995. Energy transfer in spectrally inhomogeneous light-harvesting pigment-protein complexes of purple bacteria. *Biophys. J.* 69:2211–2225.
- Hess, S., K. J. Visscher, T. Pullerits, V. Sundström, G. J. S. Fowler, and C. N. Hunter. 1994. Enhanced rates of subpicosecond energy transfer in the blue-shifted light harvesting LH2 mutants of *Rhodobacter sphaeroides*. *Biochemistry*. 33:8300–8305.
- Jimenez, R., S. N. Dikshit, S. E. Bradforth, and G. R. Fleming. 1996. Electronic excitation transfer in the LH2 complex of *Rhodobacter sphaeroides*. *J. Phys. Chem.* 100:6825–6834.
- Kennis, J. T. M., A. M. Streltsov, S. T. E. Vulto, T. J. Aartsma, T. Nozowa, and J. Amesz. 1997. Femtosecond dynamics in isolated LH2 complexes of various species of purple bacteria. *J. Phys. Chem. B*. 101:7827–7834.
- Koolhaas, M. H. C., R. N. Frese, G. J. S. Fowler, T. S. Bibby, S. Georgakopoulou, G. van der Zwan, C. N. Hunter, and R. van Grondelle. 1998. Identification of the upper exciton component of the B850 bacteriochlorophylls of the LH2 antenna complex, using a B800-free mutant of *Rhodobacter sphaeroides*. *Biochemistry*. 37:4693–4698.
- Krueger, B. P., G. D. Scholes, and G. R. Fleming. 1998. Calculation of couplings and energy-transfer pathways between the pigments of LH2 by the *ab initio* transition density cube method. *J. Phys. Chem. B*. 102:5378–5386.
- Krueger, B. P., G. D. Scholes, I. R. Gould, and G. R. Fleming. 1999. Carotenoid mediated B800–B850 coupling in LH2. *PhysChemComm* 8 (<http://www.rsc.org/ej/qu/1999/c9903172>).
- Kühn, O., and V. Sundström. 1997. Energy transfer and relaxation dynamics in light-harvesting antenna complexes of photosynthetic bacteria. *J. Phys. Chem. B*. 101:3432–3440.
- Leupold, D., H. Stiel, J. Ehlert, F. Nowak, K. Teuchner, B. Voigt, M. Bandilla, B. Ücker, and H. Scheer. 1999. Photophysical characterization of the B800-depleted light harvesting complex B850 of *Rhodobacter sphaeroides*. Implications to the ultrafast energy transfer. 800–850 nm. *Chem. Phys. Lett.* 301:537–545.
- Ma, Y.-Z., R. J. Cogdell, and T. Gillbro. 1997. Energy transfer and exciton annihilation in the B800–850 antenna complex of the photosynthetic purple bacterium *Rhodospseudomonas acidophila* (strain 10050). A femtosecond transient absorption study. *J. Phys. Chem. B*. 101:1087–1095.
- McDermott, G., S. M. Prince, A. A. Freer, A. M. Hawthornthwaite-Lawless, M. Z. Papiz, R. J. Cogdell, and N. W. Isaacs. 1995. Crystal structure of an integral membrane light-harvesting complex from photosynthetic bacteria. *Nature*. 374:517–521.
- Monshouwer, R., I. O. de Zafra, F. van Mourik, and R. van Grondelle. 1995. Low-intensity pump-probe spectroscopy on the B800 to B850 transfer in the light harvesting 2 complex of *Rhodobacter sphaeroides*. *Chem. Phys. Lett.* 246:341–346.
- Moog, R. S., A. Kuki, M. D. Fayer, and S. G. Boxer. 1984. Excitation transport and trapping in a synthetic chlorophyllide substituted hemoglobin: orientation of the chlorophyll S₁ transition dipole. *Biochemistry*. 23:1564–1571.
- Peterman, E. J. G., T. Pullerits, R. van Grondelle, and H. van Amerongen. 1997. Electron-phonon coupling and vibronic fine structure of light-harvesting complex II of green plants: temperature dependent absorption and high-resolution fluorescence spectroscopy. *J. Phys. Chem. B*. 101: 4448–4457.
- Pfennig, N. 1969. *Rhodospseudomonas acidophila*, a new species of the budding purple non-sulfur bacteria. *J. Bacteriol.* 99:597–602.
- Pullerits, T., S. Hess, J. L. Herek, and V. Sundström. 1997. Temperature dependence of excitation transfer in LH2 of *Rhodobacter sphaeroides*. *J. Phys. Chem. B*. 101:10560–10567.
- Renge, I., K. Mauring, and R. Avarmaa. 1987. Site-selection optical spectra of bacterio-chlorophyll and bacteriopheophytin in frozen solutions. *J. Luminescence*. 37:207–214.
- Sauer, K., R. J. Cogdell, S. M. Prince, A. A. Freer, N. W. Isaacs, and H. Scheer. 1996. Structure-based calculations of the optical spectra of the LH2 bacteriochlorophyll-protein complex from *Rhodospseudomonas acidophila*. *Photochem. Photobiol.* 64:564–576.
- Scholes, G. D., I. R. Gould, R. J. Cogdell, and G. R. Fleming. 1999. *Ab initio* molecular orbital calculations of electronic couplings in the LH2 bacterial light-harvesting complex of *Rps. acidophila*. *J. Phys. Chem. B*. 103:2543–2553.
- Scholes, G. D., R. D. Harcourt, and G. R. Fleming. 1997. Electronic interactions in photosynthetic light-harvesting complexes: the role of carotenoids. *J. Phys. Chem. B*. 101:7302–7312.
- Shreve, A. P., J. K. Trautman, H. A. Frank, T. G. Owens, and A. C. Albrecht. 1991. Femtosecond energy-transfer processes in the B800–850 light-harvesting complex of *Rhodobacter sphaeroides* 2.4.1. *Biochim. Biophys. Acta*. 1058:280–288.
- Sumi, H. 1999. Theory on rates of excitation-energy transfer between molecular aggregates through distributed transition dipoles with application to the antenna system in bacterial photosynthesis. *J. Phys. Chem. B*. 103:252–260.
- Sundström, V., T. Pullerits, and R. van Grondelle. 1999. Photosynthetic light-harvesting: reconciling dynamics and structure of purple bacterial LH2 reveals function of photosynthetic unit. *J. Phys. Chem. B*. 103: 2327–2346.
- Tars, M., A. Ellervee, P. Kuk, A. Laisaar, A. Saarnak, and A. Freiberg. 1994. Photosynthetic proteins under high pressure. *Lithuanian J. Phys.* 34:320–328.
- Teuchner, K., H. Stiel, D. Leupold, I. Katheder, and H. Scheer. 1994. From chlorophyll *a* towards bacteriochlorophyll *a*: excited-state processes of modified pigments. *J. Luminescence*. 60–61:520–522.
- Teuchner, K., H. Stiel, D. Leupold, A. Scherz, D. Noy, I. Simonin, G. Hartwich, and H. Scheer. 1997. Fluorescence and excited state absorption in modified pigments of bacterial photosynthesis: a comparative study of metal-substituted bacteriochlorophylls *a*. *J. Luminescence*. 72–74:612–614.
- Todd, J. B., P. S. Parkes-Loach, J. F. Leykam, and P. A. Loach. 1998. In vitro reconstitution of the core and peripheral light-harvesting complexes of *Rhodospirillum rubrum* from separately isolated components. *Biochemistry*. 37:17458–17468.
- van Grondelle, R., J. P. Dekker, T. Gillbro, and V. Sundström. 1994. Energy transfer and trapping in photosynthesis. *Biochim. Biophys. Acta*. 1187:1–65.
- Wu, H.-M., M. Ratsep, R. Jankowiak, R. J. Cogdell, and G. J. Small. 1997. Comparison of the LH2 antenna complexes of *Rhodospseudomonas acidophila* (strain 10050) and *Rhodobacter sphaeroides* by high-pressure absorption, high-pressure hole burning, and temperature-dependent absorption spectroscopies. *J. Phys. Chem. B*. 101:7641–7653.
- Wu, H.-M., S. Savikhin, N. R. S. Reddy, R. Jankowiak, R. J. Cogdell, W. S. Struve, and G. J. Small. 1996. Femtosecond and hole-burning studies of B800's excitation energy relaxation dynamics in the LH2 antenna complex of *Rhodospseudomonas acidophila* (Strain 10050). *J. Phys. Chem.* 100:12022–12033.